SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 10, 12 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

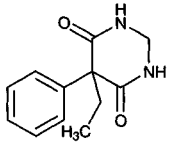
CHROMATOGRAM
Retention time: 35.17, 39.67

OTHER SUBSTANCES
Simultaneous: baclofen, levodopa, norephedrine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Primidone



Molecular formula: $C_{12}H_{14}N_2O_2$
Molecular weight: 218.26
CAS Registry No.: 125-33-7
Merck Index: 7927
Lednicer No.: 1 276

SAMPLE
Matrix: blood

Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, phenytoin

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 3.0
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, phenobarbital, phenytoin, carbamazepine, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL heptabarbital in MeOH + 500 μL 400 mM pH 7.0 sodium phosphate buffer + 10 mL ethyl acetate, extract. Evaporate the extract to dryness at 50°, reconstitute the residue in 20 μL MeOH, inject a 3 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 Whatman Co:Pell ODS

Column: 125 × 4.5 5 μm SAS Hypersil
Mobile phase: MeCN:buffer 20:80 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 7.5 with phosphoric acid.)
Flow rate: 1.6
Injection volume: 3
Detector: UV 200

CHROMATOGRAM
Retention time: 4.2
Internal standard: heptabarbital (9.8)
Limit of quantitation: 2.5 μM

OTHER SUBSTANCES
Extracted: ethosuximide, phenobarbital, pheneturide, carbamazepine, phenytoin
Simultaneous: phenylethylmalonamide, sulthiame, sulfamethoxazole, ethotoin, butabarbital, pentobarbital, methsuximide, cyclobarbital, ethylphenacemide, amobarbital, glutethimide, secobarbital, barbital

---

KEY WORDS
plasma; horse

REFERENCE
Christofides,J.A.; Fry,D.E. Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography, *Clin.Chem.*, **1980**, *26*, 499–501.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 20 μg/mL IS in MeOH:water 10:90 + 75 μL glacial acetic acid, vortex for 30 s, add 5 mL chloroform, shake for 5 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 Permaphase ETH (DuPont)
Column: 250 × 4.6 CLC 1 C8 (DuPont)
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM KH_2PO_4 and 1 mM K_2HPO_4 adjusted to pH 5.6.)
Column temperature: 25
Flow rate: 2
Injection volume: 40
Detector: UV 220

CHROMATOGRAM
Retention time: 2.0
Internal standard: alphenal (5-allyl-5-phenylbarbituric acid) (4.4)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: ethosuximide, phenytoin, carbamazepine, phenobarbital
Simultaneous: amobarbital, barbital, chlordiazepoxide, codeine, cortisol, ethotoin, glutethimide, hexobarbital, mephenytoin, mephobarbital, metharbital, methsuximide, nitrazepam, pentobarbital, phenacetin, phensuximide, secobarbital
Noninterfering: acetaminophen, acetazolamide, amphetamine, bilirubin, caffeine, diazepam, dimenhydrinate, meperidine, meprobamate, methamphetamine, methaqualone, methylphenidate, nicotine, propoxyphene, theophylline, valproate

---

KEY WORDS
plasma; serum

REFERENCE
Rydzewski,R.S.; Gadsden,R.H.; Phelps,C.A. Simultaneous rapid HPLC determination of anticonvulsant drugs in plasma and correlation with EMIT, *Ann.Clin.Lab.Sci.*, **1980**, *10*, 89–94.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 10.0
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS
serum

REFERENCE
Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Serum or plasma + 400 μL 10 μg/mL IS in acetone, vortex for 10 s, centrifuge at 4500-5000 g for 1 min, remove the supernatant to another tube, centrifuge for 30 s, inject a 5-7.5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:buffer 17:28:55, final pH 6.8-7.0 (Buffer was 400 μL 1 M KH_2PO_4 in 1 L water, pH adjusted to 6.0 with 900 mM phosphoric acid.)
Column temperature: 30
Flow rate: 0.7
Injection volume: 5-7.5
Detector: UV 195

CHROMATOGRAM
Retention time: 7.4
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (13.8)

OTHER SUBSTANCES
Extracted: carbamazepine, N-desmethylmethsuximide, ethosuximide, phenobarbital, phenytoin
Simultaneous: acetaminophen, butalbital, caffeine, hexobarbital, methsuximide, phenacetin, phenylethylmalonamide, salicylic acid

KEY WORDS
plasma; serum

REFERENCE
Szabo,G.K.; Browne,T.R. Improved isocratic liquid-chromatographic simultaneous measurement of phenytoin, phenobarbital, primidone, carbamazepine, ethosuximide, and N-desmethylmethsuximide in serum, *Clin.Chem.*, **1982**, *28*, 100–104.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 10 μg/mL IS in MeCN, vortex for 10 s, centrifuge at 3000 g for 1 min, remove the supernatant and place it in another tube, centrifuge for 1 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 5 μm Nova Pak C18 Radial pak
Mobile phase: MeCN:MeOH:acetone:buffer 8:21:10:61 adjusted to pH 7.95 ± 0.02 with NaOH (Buffer was 1.36 g/L KH_2PO_4.)
Flow rate: 2.8
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 2.03
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (4.89)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: ethosuximide, phenobarbital, carbamazepine, phenytoin, metabolites
Simultaneous: acetaminophen, N-acetylprocainamide, aspirin, ampicillin, caffeine, cephapirin, chloramphenicol, digoxin, disopyramide, hexobarbital, indomethacin, lidocaine, mephobarbital, methsuximide, nafcillin, pentobarbital, phenylethylmalonamide, procainamide, quinidine, salicylic acid, secobarbital, sulfamerazine, sulfamethazine, terbutaline, tetracycline, theobromine, theophylline
Noninterfering: acetazolamide, amikacin, cephalosporin C, gentamicin, propranolol, sulfadiazine, sulfamethoxazole, sulfisoxazole, tobramycin, valproic acid, verapamil

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Rognerud,C.L. Simultaneous measurement of ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine, and their bioactive metabolites by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1667–1670.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 200 μL 1 M HCl saturated with ammonium sulfate, vortex for 20 s, add 60 μL 10 μg/mL 4-methylprimidone in MeCN, vortex for 20 s, centrifuge at 2700 g for 5 min, inject a 5-10 μL aliquot of the MeCN layer.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeOH:THF:50 mM pH 5.9 phosphate buffer 44:1:55
Column temperature: 50
Flow rate: 1.1
Injection volume: 5-10
Detector: UV 210

CHROMATOGRAM
Retention time: 3
Internal standard: 4-methylprimidone (5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenobarbital, phenytoin, valproic acid
Simultaneous: acetaminophen, salicylic acid, ethylphenylmalonamide, theophylline, caffeine, ethosuximide, chloramphenicol, methylphenobarbital, glutethimide, pentobarbital, lidocaine, diazepam

KEY WORDS
plasma

REFERENCE
Kushida,K.; Ishizaki,T. Concurrent determination of valproic acid with other antiepileptic drugs by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 131–139.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 1 mL 5 μg/mL 5-ethyl-5-tolylhydantoin in MeCN, agitate for 3 min. Remove the supernatant and evaporate it to dryness, dissolve the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: pellicular reversed phase (Chrompack 28653)
Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)
Mobile phase: MeCN:50 mM NaH_2PO_4 25:75 adjusted to pH 2.2 with phosphoric acid
Flow rate: 0.9
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 1.7
Internal standard: 5-ethyl-5-tolylhydantoin (7.8)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital, ethylphenylmalonamide

KEY WORDS
serum

REFERENCE
Van Damme,M.; Molle,L.; Abi Khalil,F. Useful sample handlings for reversed phase high performance liquid chromatography in emergency toxicology, *J.Toxicol.Clin.Toxicol.*, **1985**, *23*, 589–614.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 2.71

Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, secobarbital, theophylline, thiopental

Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, procainamide, salicylamide, salicylic acid, sulfamethoxazole, tolmetin, trimethoprim, vancomycin

Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

Interfering: sulindac, phenylbutazone

KEY WORDS

serum

REFERENCE

Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE

Matrix: blood

Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM

Retention time: 1.61
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, caffeine, barbital, ethosuximide, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone

Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS

plasma; SPE

REFERENCE

Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 2 μg 10-methoxycarbamazepine + 25 μL 1 M NaOH + 1.2 mL dichloromethane, mix for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 20 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.9 10 μm LiChrosorb RP8
Mobile phase: MeCN:water 32:68
Flow rate: 1.8
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 2.7
Internal standard: 10-methoxycarbamazepine (9.3)

OTHER SUBSTANCES
Extracted: carbamazepine, oxcarbazepine, phenobarbital
Noninterfering: clobazam, clonazepam, diazepam, ethosuximide, phenytoin, valproic acid

KEY WORDS
plasma

REFERENCE
Elyas,A.A.; Goldberg,V.D.; Patsalos,P.N. Simple and rapid micro-analytical high-performance liquid chromatographic technique for the assay of oxcarbazepine and its primary active metabolite 10-hydroxycarbazepine, *J.Chromatogr.*, **1990**, *528*, 473–479.

SAMPLE
Matrix: blood
Sample preparation: Inject 20 μL serum onto column A with mobile phase A and elute to waste, after 1.5 min backflush the contents of column A onto column B with mobile phase B, after 1 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 30 × 4.6 ISRP silica (for preparation see Anal. Chem. 1989, 61, 2445); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: A 14 mM NaH_2PO_4 containing 6 mM Na_2HPO_4; B MeCN:MeOH:14 mM NaH_2PO_4 containing 6 mM Na_2HPO_4 15:20:65
Flow rate: 0.8
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenobarbital, phenytoin

KEY WORDS
serum; column-switching

REFERENCE
Haginaka,J.; Wakai,J.; Yasuda,H.; Kimura,Y. Determination of anticonvulsant drugs and methyl xanthine derivatives in serum by liquid chromatography with direct injection: column-switching method using a new internal-surface reversed-phase silica support as a precolumn, *J.Chromatogr.*, **1990**, *529*, 455–461.

SAMPLE
Matrix: blood
Sample preparation: Add two volumes of MeCN to the mouse serum, mix, centrifuge at 1500 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Sentry (Waters)
Column: 150 × 4.6 Nova-Pak C18
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110
Column temperature: 40
Flow rate: 0.5
Injection volume: 5
Detector: UV 214

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Extracted: phenylethyl malonamide, phenobarbital, carbamazepine, phenytoin, carbamazepine-10,11-epoxide

KEY WORDS
serum; mouse

REFERENCE
Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE
Matrix: blood, saliva, urine
Sample preparation: Serum. 100 μL Serum + 200 μL MeCN, vortex for 10 s, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Saliva. 250 μL Saliva + 50 μL MeCN, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Urine. Condition a Sep-Pak SPE cartridge with 5 mL MeCN then 20 mL water. Add 2 mL urine to the cartridge, wash with 20 mL water, elute with 500 μL MeCN, inject 2 μL of the eluent.

HPLC VARIABLES
Guard column: 20 × 2 3 μm ODS-Hypersil
Column: 250 × 2 3 μm ODS-Hypersil
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110
Column temperature: 40
Flow rate: 0.2
Injection volume: 2
Detector: UV 200

CHROMATOGRAM
Retention time: 5.0
Limit of quantitation: 780 ng/mL

OTHER SUBSTANCES
Simultaneous: p-hydroxyphenobarbital, phenylethylmaleimide, phenobarbital, dihydrodihydroxycarbamazepine, 5-(p-hydroxyphenyl)-5-phenylhydantoin, carbamazepine, 5-(m-hydroxyphenyl)-5-phenylhydantoin, phenytoin, carbamazepine-10,11-epoxide, hexobarbital, nitrazepam, clonazepam
Noninterfering: oxazepam, nordiazepam, cyheptamide, diazepam, prezepam, temazepam, lorazepam, chlordiazepoxide

KEY WORDS
serum; SPE

REFERENCE
Liu,H.; Delgado,M.; Forman,L.J.; Eggers,C.M.; Montoya,J.L. Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1993**, *616*, 105–115.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize 20-200 mg brain tissue with 1 mL 1.5 μg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1, flush apparatus with 1 mL extraction buffer, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 μL MeOH, add 50 μL water, inject a 10-25 μL aliquot. Serum. 100 μL Serum + 1 mL 1.5 μg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1,mix, add 1 mL extraction buffer, mix, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 μL MeOH, add 50 μL water, inject a 10-25 μL aliquot. (Extraction buffer was 20 g $NaH_2PO_4.2H_2O$ + 4.5 g $Na_2HPO_4.2H_2O$ + 1.5 NaN_3 in 1 L water, pH 6. Extraction solvent was dichloromethane:isopropanol 97:3.)

HPLC VARIABLES
Column: 200 × 2.1 5 μm Hypersil ODS
Mobile phase: Gradient. A was MeCN:50 mM $(NH_4)H_2PO_4$ (pH 4.4) 10:90. B was MeCN:50 mM $(NH_4)H_2PO_4$ (pH 4.4) 60:40. A:B from 85:15 to 55:45 over 9.5 min, keep at 55:45 for 0.5 min, return to 85:15 over 0.5 min.
Column temperature: 65
Flow rate: 0.3
Injection volume: 10-25
Detector: UV 207

CHROMATOGRAM
Retention time: 3.73
Internal standard: 5-ethyl-5-(p-tolyl)barbituric acid (9.07)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital, N-desmethylmethsuximide, carbamazepine-10,11-epoxide, phenytoin, carbamazepine

KEY WORDS
serum; SPE; brain

REFERENCE
Juergens,U.; Rambeck,B. Sensitive analysis of antiepileptic drugs in very small portions of human brain by microbore HPLC, *J.Liq.Chromatogr.*, **1987**, *10*, 1847–1863.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Inject a 5 or 20 μL aliquot directly onto the column with mobile phase A or C. Urine. Inject a 5 μL aliquot directly onto the column with mobile phase C.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 10:90 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer 14:86 for 5 min, to 25:75 over 1 min, to 30:70 over 2 min, to 50:50 over 3 min, maintain at 50:50 for 6 min (C)
Flow rate: 1
Injection volume: 5 (A, C), 20 (B)
Detector: UV 254 (serum); UV 230 (urine)

CHROMATOGRAM
Retention time: 2.16 (A, serum), 9.7 (B, serum), 4.5 (C, urine)
Limit of detection: 1 ng (urine)

OTHER SUBSTANCES
Extracted: acetaminophen (B), barbital (B), carbamazepine (B,C), ethosuximide (A), methamphetamine (A), phenobarbital (B,C), phenytoin (B,C), sulfamethoxazole (A), sulfapyridine (B)
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.13

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30

Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Pristinamycin

Merck Index: 7933

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 227.5

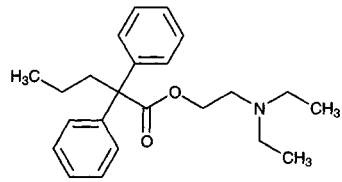
CHROMATOGRAM
Retention time: 17.235

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Proadifen

Molecular formula: $C_{23}H_{31}NO_2$
Molecular weight: 353.50
CAS Registry No.: 302-33-0, 62-68-0 (HCl)



SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

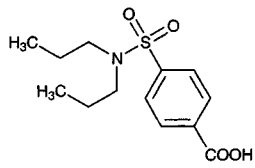
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cy-

clizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Probenecid



Molecular formula: $C_{13}H_{19}NO_4S$
Molecular weight: 285.36
CAS Registry No.: 57-66-9
Merck Index: 7934
Lednicer No.: 1 135

SAMPLE

Matrix: blood
Sample preparation: Filter plasma (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm GFF-S5-80 internal-surface reversed phase "Pinkerton" (Regis)
Mobile phase: THF:100 mM potassium phosphate 5:95, pH 7.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7.8

KEY WORDS

plasma; direct injection

REFERENCE

Nakagawa,T.; Shibukawa,A.; Shimono,N.; Kawashima,T.; Tanaka,H.; Haginaka,J. Retention properties of internal-surface reversed-phase silica packing and recovery of drugs from human plasma, *J.Chromatogr.*, **1987**, *420*, 297–311.

SAMPLE

Matrix: blood

Sample preparation: 50 μL Plasma + 350 μL MeOH, mix thoroughly, centrifuge at 10000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeCN:20 mM pH 7.5 phosphate buffer 25:75
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Limit of quantitation: 1000 ng/mL

KEY WORDS

plasma

REFERENCE

Terashita,S.; Sawamoto,T.; Deguchi,S.; Tokuma,Y.; Hata,T. Sex-dependent and independent renal excretion of nilvadipine metabolites in rat: evidence for a sex-dependent active secretion in kidney, *Xenobiotica*, **1995**, *25*, 37–47.

SAMPLE

Matrix: blood

Sample preparation: 150 μL Plasma + 150 μL MeCN, vortex, rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min. Transfer supernatant to another tube and add 7 volumes dichloromethane, equilibrate for 10 min, rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min, inject an aliquot of the upper aqueous layer (J.Chromatogr. 1987, 413, 109).

HPLC VARIABLES

Guard column: C18
Column: 150 × 1.6 Spherisorb S5-ODS2 C18
Mobile phase: MeOH:100 mM pH 3 acetate buffer 50:50
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Limit of detection: 300 ng/mL

KEY WORDS

plasma; rat

REFERENCE

Gimeno,M.J.; Martínez,M.; Granero,L.; Torres-Molina,F.; Peris,J.-E. Influence of probenecid on the renal excretion mechanisms of cefadroxil, *Drug Metab.Dispos.*, **1996**, *24*, 270–272.

SAMPLE

Matrix: blood, CSF

Sample preparation: Mix 100 μL plasma or 50 μL CSF with an equal volume of 5 μg/mL n-butyl p-hydroxybenzoate in MeCN, centrifuge at 14000 rpm, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4 LiChrospher RP-18e

Mobile phase: MeCN:water:acetic acid 50:49.9:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 244

CHROMATOGRAM
Internal standard: n-butyl p-hydroxybenzoate

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Seki,T.; Sato,N.; Hasegawa,T.; Kawaguchi,T.; Juni,K. Nasal absorption of zidovudine and its transport to cerebrospinal fluid in rats, *Biol.Pharm.Bull.*, **1994**, *17*, 1135–1137.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Plasma. Add 500 μL MeCN to 500 μL plasma while mixing on a Whirlmixer, rotate for 10 min, centrifuge at 1000 g for 5 min. Remove a 700 μL aliquot of the supernatant and ad it to 3.5 mL dichloromethane, mix for 30 s, centrifuge at 1000 g for 1 min, inject a 20 μL aliquot of the aqueous layer. Urine. Dilute with Sörensen buffer, inject an aliquot. CSF. Inject an aliquot directly.

HPLC VARIABLES
Column: 100 × 3 5 μm MOS-Hypersil C8
Mobile phase: MeCN:MeOH:buffer 12:26:62 containing 3 mM tetrabutylammonium bromide (Buffer was 5 mM pH 5.0 sodium acetate.)
Column temperature: 22
Flow rate: 1
Injection volume: 20
Detector: UV 231

CHROMATOGRAM
Retention time: 11
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: penicillin G

KEY WORDS
plasma

REFERENCE
van Gulpen,C.; Brokerhof,A.W.; van der Kaay,M.; Tjaden,U.R.; Mattie,H. Determination of benzylpenicillin and probenecid in human body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *381*, 365–372.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 350 μL 2 μg/mL Naproxen in 10 mM pH 6.0 phosphate buffer containing 0.05% MeOH + 650 μL pH 6 phosphate buffer + 100 μL plasma + 0.5 mL 1 M pH 2 phosphate buffer + 10 mL diethyl ether, vortex 1 min, centrifuge at 2000 g for 3 min. Remove organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 250 μL mobile phase, vortex for 15 s, inject aliquot. Urine. 350 μL 20 μg/mL Naproxen in 10 mM pH 6.0 phosphate buffer containing 0.5% MeOH + 650 μL pH 6 phosphate buffer + 100 μL urine + 1 mL 0.5 M pH 7 phosphate buffer + 10 mL diethyl ether, vortex 1 min, centrifuge at 2000 g for 3 min. Remove organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 250 μL mobile phase, vortex for 15 s, inject aliquot.

HPLC VARIABLES
Guard column: 40 × 3.2 30-44 μm Vydac reverse-phase

Column: 40 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 6:94 to 8:92
Flow rate: 2
Injection volume: 5-200
Detector: UV 262

CHROMATOGRAM
Retention time: 18
Internal standard: naproxen (10)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: ketoprofen, fenoprofen, salicylic acid

KEY WORDS
plasma

REFERENCE
Upton,R.A.; Buskin,J.N.; Guentert,T.W.; Williams,R.L.; Riegelman,S. Convenient and sensitive high-performance liquid chromatography assay for ketoprofen, naproxen and other allied drugs in plasma or urine, *J.Chromatogr.*, **1980**, *190*, 119–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 100 μL 100 μg/mL indoprofen in water + 100 μL 600 mM sulfuric acid + 5 mL isooctane:isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and add 5 mL water to it. Vortex for 30 s, centrifuge for 3 min. Remove organic layer and evaporate it to dryness on a Speed Vac concentrator. Reconstitute residue in 100 μL 50 mM triethylamine in MeCN, vortex 30 s, add 50 μL 60 mM ethyl chloroformate in MeCN, let stand 30 s, add 50 μL 1 M L-leucinamide hydrochloride and 1 M triethylamine in MeOH, let stand 2 min, add 50 μL water, inject 10-60 μL aliquots. Urine. 100 μL Urine + 25 μL 1 M NaOH, add 125 μL 600 mM sulfuric acid, proceed as for plasma.

HPLC VARIABLES
Guard column: 50 × 5 37-53 μm C18 material
Column: 100 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:60 mM KH_2PO_4:triethylamine 35:65:0.1
Flow rate: 1
Injection volume: 10-60
Detector: UV 275

CHROMATOGRAM
Retention time: 18
Internal standard: indoprofen (6(R), 7(S))
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: ketoprofen
Also analyzed: fenoprofen, cicloprofen, pirprofen, flurbiprofen, indoprofen, carprofen

KEY WORDS
plasma; rat; derivatization

REFERENCE
Palylyk,E.L.; Jamali,F. Simultaneous determination of ketoprofen enantiomers and probenecid in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *568*, 187–196.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 100 μL 600 mM sulfuric acid + 4 mL isooctane: isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and add 4

mL water to it. Vortex for 30 s, centrifuge for 3 min. Remove organic layer and evaporate it to dryness on a Speed Vac concentrator. Reconstitute residue in 200 μL MeOH, vortex 30 s, add 100 μL 100 μg/mL indoprofen in water, 20 μL aliquots. Urine. 100 μL Urine + 25 μL 1 M NaOH, add 125 μL 600 mM sulfuric acid, proceed as for plasma.

HPLC VARIABLES
Guard column: 50 × 5 37-53 μm C18 material
Column: 100 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:60 mM KH_2PO_4:triethylamine 25:75:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 6.5
Internal standard: indoprofen (3.4)

OTHER SUBSTANCES
Simultaneous: ketoprofen

KEY WORDS
plasma; rat

REFERENCE
Palylyk,E.L.; Jamali,F. Simultaneous determination of ketoprofen enantiomers and probenecid in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *568*, 187–196.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 RP-8
Mobile phase: MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na_2HPO_4 and 7 mM KH_2PO_4 to achieve pH 7.)
Flow rate: 1
Injection volume: 50
Detector: F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 μg/mL reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 m × 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetonitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α-(3,4-dimethoxyphenyl)-4'- methylcinnamonitrile. Dissolve 20 mmoles α-(3,4-dimethoxyphenyl)-4'-methylcinnamonitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h , cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile (J.Chem.Eng.Data 1987, 32, 387). Reflux 10 mmoles α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM
Retention time: k' 1.2791
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: ibuprofen, ketoprofen, mefenamic acid, naproxen, salicylic acid, valproic acid

KEY WORDS
post-column extraction; post-column reaction

REFERENCE
Kim,M.; Stewart,J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α-phenylcinnamonitrile quaternary ammonium salt as a new fluorescent ion-pair reagent, *J.Liq.Chromatogr.*, **1990**, *13*, 213–237.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinna-

mine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 7.49

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 15.78 (A), 16.1 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 5000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, spironolactone, canrenone, flumethiazide, bumetanide, ethacrynic acid

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 15:15:70:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 3.2

Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES

Extracted: bumetanide, ethacrynic acid, spironolactone

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 19.9
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN: water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 7.3
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, clopamide, chlorthalidone, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, spironolactone, torsemide, triamterene

REFERENCE
Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE
Matrix: urine
Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

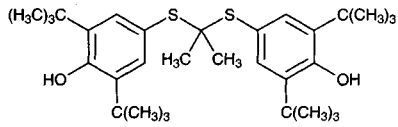
CHROMATOGRAM
Retention time: 10.3
Limit of detection: 200 ng/mL.

OTHER SUBSTANCES
Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, spironolactone, triamterene

KEY WORDS
column-switching

REFERENCE
Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Probucol



Molecular formula: $C_{31}H_{48}O_2S_2$
Molecular weight: 516.85
CAS Registry No.: 23288-49-5
Merck Index: 7935
Lednicer No.: 2 126

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL 10 μg/mL IS in EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil

Mobile phase: MeCN:water:THF 81.3:5.7:13
Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 244

CHROMATOGRAM
Retention time: 2.763
Internal standard: 2-pentanone bis(3,5-di-tert)mercaptole (3.666)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol), gamma-tocopherol, vitamin A (retinol), lycopene, α-carotene, β-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 400 μL 20 μg/mL IS in EtOH + 800 μL isooctane + 500 μL water, vortex for 20 s, centrifuge at 960 g for 5 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL MeCN, inject a 10 μL aliquot.

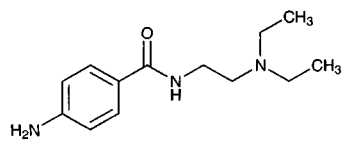
HPLC VARIABLES
Column: 200 × 3 5 μm 5 μm Hypersil ODS
Mobile phase: MeCN:water 96:4
Flow rate: 1
Injection volume: 10
Detector: UV 241

CHROMATOGRAM
Retention time: 5
Internal standard: 4,4'-[1-methylbutylidene-bis(thio)]-bis[2,6-bis(1,1-dimethylethyl)]phenol (MDL 27272) (7)
Limit of detection: 500 ng/mL

KEY WORDS
plasma; rabbit

REFERENCE
Nourooz-Zadeh,J.; Gopaul,N.K.; Forster,L.A.; Ferns,G.A.; Änggård,E.E. Measurement of plasma probucol levels by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 55–60.

Procainamide



Molecular formula: $C_{13}H_{21}N_3O$
Molecular weight: 235.33
CAS Registry No.: 51-06-9, 614-39-1 (HCl)
Merck Index: 7936
Lednicer No.: 1 14

SAMPLE
Matrix: activated neutrophils
Sample preparation: Centrifuge, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 mm long 5 μm Spherisorb ODS2
Mobile phase: MeCN:water:acetic acid:triethylamine 20:80:1:0.05
Flow rate: 1
Injection volume: 100
Detector: UV

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Uetrecht,J.P.; Zahid,N.; Whitfield,D. Metabolism of vesnarinone by activated neutrophils: Implications for vesnarinone-induced agranulocytosis, *J.Pharmacol.Exp.Ther.*, **1994**, *270*, 865–872.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL buffer + 50 μL 10 μg/mL N-propionylprocainamide in MeCN + 3 mL chloroform:isopropanol 95:5, mix for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot. (Buffer was 2.9 g/L sodium bicarbonate in water, adjusted to pH 11.0 with NaOH.)

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH_2PO_4 adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean with water for 20 min and MeOH for 30 min.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.8
Internal standard: N-propionylprocainamide (6)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: N-acetylprocainamide
Simultaneous: dyphylline, theophylline, caffeine, aspirin, salicylic acid, acetaminophen
Noninterfering: benzoic acid

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Frawley,V.L. Theophylline, dyphylline, caffeine, acetaminophen, salicylate, acetylsalicylate, procainamide, and N-acetylprocainamide determined in serum with a single liquid-chromatographic assay, *Clin.Chem.*, **1982**, *28*, 2157–2160.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 5 mL MTBE, place on a reciprocating shaker at low speed for 15 min, centrifuge at 1250 g for 10 min. Remove organic layer and evaporate under a stream of nitrogen. Reconstitute in 200 μL mobile phase, inject 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelco Pelliguard C18
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:7 mM sodium heptanesulfonate 18:82, containing 1% glacial acetic acid and 0.035% triethylamine
Flow rate: 0.8
Injection volume: 100
Detector: UV 272

CHROMATOGRAM
Retention time: 10.1
Internal standard: procainamide.HCl

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide
Noninterfering: aspirin, acetaminophen, ibuprofen

KEY WORDS
plasma; procainamide is IS

REFERENCE
Azumaya,C.T. Sensitive liquid chromatographic method for the determination of hydrochlorothiazide in human plasma, *J.Chromatogr.*, **1990**, *532*, 168–174.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum or plasma + 100 μL 500 mM sodium carbonate + 100 μL 15 μg/mL N-propionylprocainamide in water, vortex for 5 s, add 0.5 (procainamide) or 1 (tocainide) mL dichloromethane, vortex for 30 (procainamide) or 60 (tocainide) s, centrifuge at 9500 g for 1 min. Remove the lower organic layer and add it to 200 μL 10 mM HCl, vortex for 15 s, centrifuge, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 100 × 5 NovaPak cyano HP radial compression
Mobile phase: MeCN:buffer 10:90, final pH adjusted to 6.0 (Buffer was 5 mM acetate buffer containing 0.05% triethylamine.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3.7
Internal standard: N-propionylprocainamide (6.1)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: N-acetylprocainamide, tocainide
Simultaneous: disopyramide, lidocaine, mexiletine, quinidine
Noninterfering: carbamazepine, desmethyldoxepin, digoxin, doxepin, ethosuximide, lithium, phenobarbital, phenytoin, primidone, propranolol, theophylline, valproic acid

KEY WORDS
serum; plasma

REFERENCE
vasBinder,E.; Annesley,T. Liquid chromatographic analysis of mexiletine in serum, with alternate application to tocainide, procainamide, and N-acetylprocainamide, *Biomed.Chromatogr.*, **1991**, *5*, 19–22.

SAMPLE
Matrix: blood

Sample preparation: Condition a 100 mg LRC Bond Elut unendcapped cyanopropyl SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Serum or plasma + 250 μL buffer, vortex, centrifuge at 1300 rpm for 10 min (plasma only), add 1 mL sample to SPE cartridge, wash twice with 1 mL water, dry with nitrogen for 30 s, elute with two 250 μL aliquots of MeOH: buffer 60:40, inject 100 μL aliquot of buffer. (Buffer was 50 mM KH_2PO_4 and Na_2HPO_4 50:50 v/v adjusted to pH 6.0 with phosphoric acid.)

HPLC VARIABLES
Guard column: 15 × 4.6 7 μm Brownlee RP-18
Column: 250 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 50 mM KH_2PO_4 and Na_2HPO_4 50:50 v/v adjusted to pH 6.0 with phosphoric acid.)
Column temperature: 48
Flow rate: 1.25
Injection volume: 100
Detector: UV 320

CHROMATOGRAM
Retention time: 5.4
Internal standard: procainamide

OTHER SUBSTANCES
Simultaneous: ranitidine

KEY WORDS
serum; plasma; robotic sample preparation; procainamide is IS; SPE

REFERENCE
Lloyd,T.L.; Perschy,T.B.; Gooding,A.E.; Tomlinson,J.J. Robotic solid phase extraction and high performance liquid chromatographic analysis of ranitidine in serum or plasma, *Biomed.Chromatogr.*, **1992**, *6*, 311–316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 287

CHROMATOGRAM
Retention time: 3.65
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihy-

dralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 25 μL MeOH + 15 μL 6 M NaOH + 2 mL ethyl acetate: isopropanol 96:4, shake mechanically for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeOH, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM pH 5.1 KH_2PO_4 8:92
Flow rate: 2.5
Injection volume: 75
Detector: UV 330

CHROMATOGRAM

Retention time: 3
Internal standard: procainamide

OTHER SUBSTANCES

Extracted: ranitidine
Simultaneous: lidocaine
Noninterfering: brompheniramine, chlorpheniramine, cimetidine, diazepam, diclofenac, glyburide, ibuprofen, ketoprofen, metoclopramide, naproxen, phenylbutazone, verapamil

KEY WORDS

plasma; procainamide is IS

REFERENCE

al-Khamis,K.I.; El-Sayed,Y.M.; Al-Rashood,K.A.; Bawazir,S.A. High-performance liquid chromatographic determination of ranitidine in human plasma, *J.Liq.Chromatogr.*, **1995**, *18*, 277–286.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 2 mL Plasma + 100 μL water + 660 μL 2 M perchloric acid, shake briefly, centrifuge at 3000 rpm for 5 min. Remove 1.5 mL of the supernatant and adjust the pH to 9 with 150 μL 4 M NaOH and 4 mL 500 mM boric acid/KCl buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase. Urine. Dilute urine with pH 9 borate buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:water:acetic acid 38:61:1 containing 1-heptanesulfonic acid (PIC B7)
Flow rate: 1.5
Injection volume: 25-100
Detector: F ex 235 em no filter

CHROMATOGRAM
Retention time: 7
Internal standard: procainamide

OTHER SUBSTANCES
Extracted: sotalol

KEY WORDS
plasma; procainamide is IS

REFERENCE
Lefebvre,M.A.; Girault,J.; Saux,M.C.; Fourtillan,J.B. Fluorometric high-performance liquid chromatographic determination of sotalol in biological fluids, *J.Pharm.Sci.*, **1980**, *69*, 1216–1217.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Sample + 9 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:500 mM KH_2PO_4:water 22:10:68 adjusted to pH 7.1 with 10 M NaOH
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: milrinone, degradation products

KEY WORDS
stability-indicating; 5% dextrose; injections

REFERENCE
Riley,C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1988**, *45*, 2079–2091.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:water:500 mM KH_2PO_4 15:75:10, pH 6.8
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: amrinone

KEY WORDS
injections; stability-indicating; 5% dextrose; 0.45% NaCl

REFERENCE
Riley,C.M.; Junkin,P. Stability of amrinone and digoxin, procainamide hydrochloride, propranolol hydrochloride, sodium bicarbonate, potassium chloride, or verapamil hydrochloride in intravenous admixtures, *Am.J.Hosp.Pharm.*, **1991**, *48*, 1245–1252.

SAMPLE
Matrix: formulations
Sample preparation: Make up 1 mL syrup to 50 mL with water. Remove a 2 mL aliquot and add it to 2 mL 0.1% procaine hydrochloride in water, make up to 100 mL with water, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil ODS-3 C18
Mobile phase: MeCN:buffer:triethanolamine:water 15:80:0.2:4.8, pH adjusted to 4.5 with glacial acetic acid (Buffer was 4.72 g sodium acetate and 1.8 mL acetic acid in 1 L water.)
Flow rate: 1.2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.6
Internal standard: procaine (7.5)

KEY WORDS
syrup; stability-indicating

REFERENCE
Alexander,K.S.; Pudipeddi,M.; Parker,G.A. Stability of procainamide hydrochloride syrups compounded from capsules, *Am.J.Hosp.Pharm.*, **1993**, *50*, 693–698.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: MeOH:21 mM pH 4.4 NaH_2PO_4 13.5:86.5
Flow rate: 1.5
Detector: UV 266

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Simultaneous: metabolites, N-acetylprocainamide

REFERENCE
Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.32 (A), 3.52 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.